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EXAMINER
NGUYEN, QUANG

ART UNIT	PAPER NUMBER
1636	19

DATE MAILED: 06/04/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/359,672	BLACKBURN ET AL.	
	Examiner	Art Unit	
	Quang Nguyen, Ph.D	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 March 2002*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-8,10-18,20,21,23-27,29,30,32-40,42-44 and 46-54* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,10-18,20,21,23-27,29,30,32-40,42-44 and 46-54* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *18*.
4) ☐ Interview Summary (PTO-413) Paper No(s). _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Applicants' amendment filed March 18, 2002 in Paper No. 17 has been entered.

Claims 1-8, 10-18, 20-21, 23-27, 29-30, 32-40, 42-44 and 46-54 are pending in the present application, and they are examined on the merits herein.

The text of those sections of Title 35 U.S.C. Code not included in this action can be found in a prior office action.

Priority

Acknowledgment is made of applicant's claim for foreign priority based on an application filed in Great Britain on 1/24/1997. It is noted, however, that applicant has not filed a certified copy of the 9701492.2 application as required by 35 U.S.C. 119(b). Accordingly, the pending claims are given to a priority date of 1/26/1998. Additionally, the filing date of PCT/GB98/00216 in the first line of the specification is wrong. It should be January 26, 1998 instead of January 24, 1997.

Claim Objections

Claims 8 and 44 objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. This is because the phrases "a mammalian cell and an avian cell" and "in mammalian or avian cells" in claims 8 and 44, respectively are not necessarily limited only to mammalian or avian ES, EC and EG cells.

Following is a new ground of rejection.

Written Description

Claims 1-8, 10-16, 29-30, 32-36, 37-40, 42-44 and 46-50 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111 (Fed. Cir. 1991), clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed.*" Vas-Cath Inc. v. Mahurkar, 19USPQ2d at 1117. The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed. "Vas-Cath Inc. v. Mahurkar, 19USPQ2d at 1116.

Applicant's invention is drawn to an *in vitro* method of obtaining a gene product by expressing a DNA in a cell selected from the group consisting of an ES cell, an EC cell and an EG cell; an *in vitro* assay for the effect of a presence of a protein or polypeptide or other product of DNA expression in the same group of cell; an *in vitro* method of assaying whether a DNA under investigation codes for a polypeptide that directs transport of a cell active protein to a cell surface in the same group of cell; DNA

sequence comprising expressing in a pluripotent cell a composite DNA; and an ES, EC or EG cell comprising the vectors of the present invention. Apart from disclosing the mouse embryonic stem (ES) MG1.19 cells, the instant specification fails to disclose the availability of ES or embryonic carcinoma (EC) derived from any other species (including mammals and birds) as encompassed by the scope of the instant claims for carrying out the present invention. Nor does the present disclosure teach the availability of any ES cell, any EC cell or any EG cell that expresses or will express a replication factor essential for the extrachromosomal replication of the second vector without requiring the presence of a first vector expressing said replication factor as encompassed by an embodiment of claims 1-8, 10-16, 33-36 and 47-48. Since an ES cell is totipotential and possesses the ability to contribute to the development of gametes to be transmitted to the next generation (Seamark, Reproduction, Fertility and Development 6:653-657, 1994, page 654; col. 2, third full paragraph; Moreadith et al., J. Mol. Med. 75:208-216, 1997; page 209, bottom of first full paragraph), at the effective filing date of the present application the accessibility and availability of true "ES" cells derived from species other than the mouse is not readily available as evidenced by the teachings of Seamark et al. (page 6, abstract), Moreadith et al. (page 214, Summary), and Mullins et al. (J. Clin. Invest. 98: S37-S40, 1996). Mullins et al. stated that "although to date chimeric animals have been generated from several species including the pig, in no species other than the mouse has germline transmission of an ES cell been successfully demonstrated" (page S38, column 1, first paragraph). The instant specification fails to disclose a representative numbers of species of true ES as well as

EC cells encompassed within the breadth of the instant claims. Furthermore, the present disclosure offers no guidance for a skilled artisan on how to obtain any ES, EC and EG cells expressing or will express a transcriptional factor essential for the extrachromosomal replication of the second vector without requiring the presence of a first vector expressing said transcriptional factor as encompassed within certain embodiment of the presently claimed invention. The claimed invention as a whole is not adequately described if the claims require essential or critical elements which are not adequately described in the specification and which are not conventional in the art as of Applicants' filing date. Possession may be shown by actual reduction to practice, clear depiction of the invention in a detailed drawing, or by describing the invention with sufficient relevant identifying characteristics such that a person skilled in the art would recognize that the inventor had possession of the claimed invention. Pfaff v. Wells Electronics, Inc., 48 USPQ2d 1641, 1646 (1998). The skilled artisan cannot envision the detailed structure of non-mouse true ES or non-mouse EC cells as well as any ES, EC and EG cells that expresses or will express a transcriptional factor essential for the extrachromosomal replication of the second vector without requiring the presence of a first vector expressing said transcriptional factor; and methods of using the same. Therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. See *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016 (Fed. Cir.

1991). One cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481, 1483.

Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on March 18, 2002 in Paper No. 17 (pages 13-14) have been fully considered.

Applicants argued that at the time the instant application was filed, embryonic stem (ES) cells from a variety of species including cow, hamster, pig, sheep and American mink were known in the art as evidenced by the cited articles of Saito et al., Doetschman et al., Notaranni et al. and Sukoyan et al. Additionally, the article of Pera et al. demonstrates that human EC was known at the effective filing date of the present application. Applicants further argued that the specification clearly describes the use of ES, EC and EG cells as indicated by line 28, page 3 to line 1 of page 4.

Applicants' arguments are respectfully found unpersuasive for the following reasons:

Firstly, none of the cited articles of Saito et al., Doetschman et al., Notaranni et al. and Sukoyan et al. reported a true non-mouse ES because none of the reported cell lines has been demonstrated to contribute to the development of gametes to be transmitted to the next generation, a property of a true ES stem cell. It is also noted that

Saito et al., Notarianni et al., Sukoyan et al. referred their cell lines as bovine embryonic cell-like cell lines, pluripotent embryonic cell lines, blastocyst-derived stem cell lines, respectively. Although Doetschman et al. reported the establishment of hamster blastocyst-derived embryonic stem cells, they also specifically stated that "We are presently carrying out experiments to determine if the hamster ES cells can colonize the germ line when introduced back into hamster blastocysts. If this proves to be possible, there will then exist a second ES cell system for small animal disease modeling" (page 227, col. 1, last paragraph). Several years after the published article of Doetschman et al., Mullins et al. stated that "although to date chimeric animals have been generated from several species including the pig, in no species other than the mouse has germline transmission of an ES cell been successfully demonstrated" (page S38, column 1, first paragraph). As such, at the effective filing date of the present application, true non-mouse ES cells were not available.

Secondly, with respect to the cited article Pera et al. which discloses the isolation and characterization a mulipotent clone of human embrynonal carcinoma cells, the reference by no means indicates that EC cells derived from numerous species encompassed by the breadth of the instant claims were available at the effective filing date of the present application for one skilled in the art to make and use the presently claimed invention.

Thirdly, none of the above cited references indicates or suggests that any ES, EC and EG cells that express or will express a replication factor essential for the

extrachromosomal replication of the second vector without requiring the presence of a first vector expressing said replication factor.

Fourthly, the simple statement found in line 28, page 3 to line 1 of page 4 "It is further preferred that the cell is an embryonic cell, in particular an ES, EC (embryonic carcinoma) or EG (embryonic gonadal) cell, or differentiated progeny of any such cell" is deemed to be sufficient teachings or an indication that Applicants have in possession the essential or critical elements to practice the full breadth of the presently claimed invention.

Accordingly, claims 1-8, 10-16, 29-30, 32-36, 37-40, 42-44 and 46-50 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth above.

Claim Rejections - 35 USC § 112

Claims 1-8, 10-16, 29-30, 32-36, 37-40, 42-44, 46-50 and 54 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for ***in vitro*** methods of obtaining a gene product by expressing a DNA in a mouse ES cell, a mouse or human EC cell or an EG cell wherein said ES, EC or EG cell is transfected with a first vector that expresses a viral replication factor, of assaying for the effect of a presence in said cells of a protein or polypeptide or other product of DNA expression or for screening a library of cDNAs or for expressing a composite DNA in said cells; the same isolated transfected mouse ES, mouse or human EC or an EG cell; an isolated cell comprising first and at least second vectors as recited, does not reasonably provide enablement for other embodiments of the claims. The specification does not enable

any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The factors to be considered in the determination of an enabling disclosure have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art and the breadth of the claims. *Ex parte Forman*, (230 USPQ 546 (Bd Pat. Appl & Unt, 1986); *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)).

The instant claims encompass a composition and *in vitro* methods of using ES cells or EC cells derived from any species, as well as any ES or EC or EG cell that expresses or will express a transcriptional factor essential for the extrachromosomal replication of the second vector without requiring the presence of a first vector expressing said transcriptional factor. The instant specification is not enabled for such a broadly claimed invention for the reasons already set forth in the Written Description section above. Moreover, with respect to the breadth of the instant claims, Applicants' attention is directed to the decision in *In re Shokal*, 113 USPQ 283 (CCPA 1957) wherein is stated:

> It appears to be well settled that a single species can rarely, if ever, afford sufficient support for a generic claim. In re Soll, 25 C.C.P.A. (Patents) 1309, 97 F.2d 623, 38 USPQ 189; In re Wahlforss et al., 28 C.C.P.A. (Patents) 867, 117 F.2d 270, 48 USPQ 397. The decisions do not however fix any definite number of species which will establish completion of a generic invention and it seems evident therefrom that such number will vary, depending on the circumstances of particular cases. Thus, in the case of small genus such as the halogens, consisting of four species, a reduction to practice of three, or perhaps even two, might serve to complete the generic invention, while in the case of a genus comprising hundreds of species, a considerably larger number of reductions to practice would probably be necessary.

Given the lack of sufficient guidance provided by the specification, it would have required undue experimentation for a skilled artisan to make and use the instant broadly claimed invention.

The instant claims also encompass the utilization of any replication factors that can support the extrachromosomal replication of second or third vectors as long as they are present in the transfected cells. The instant specification is not enabled for such a broadly claimed invention because apart from disclosing the use of viral replication factors such as polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors, SV40 large T antigen that can recognize and activate the appropriate viral origins present in second or third vectors for their maintenance and extrachromosomal replication in the transfected cells, the disclosure offers no guidance for a skilled artisan on how to make and use of any non-viral replication factors in the presently claimed invention. Since the prior arts at the effective filing date of the present application do not provide such guidance, it is incumbent upon the instant specification to do so. With the lack of sufficient guidance provided by the present disclosure, it would have required undue experimentation for a skilled artisan to make and use the full breadth of the presently claimed invention.

With respect to claim 54, as written it encompasses both an *in vitro* and an *in vivo* cell comprising the vectors of the presently claimed invention. The instant specification is not enabled for such a broadly claimed invention, because the specification fails to provide any guidance or direction for a skilled artisan on the make and use of any of such recombinant cell ***in vivo***. The specification provides teachings

exclusively on the construction of vectors and their applications in tissue cell cultures. The nature of the claim encompassing the *in vivo* scope would fall within the realm of gene therapy which at the effective filing date of the present application was immature and highly unpredictable. The instant specification fails to provide guidance for a skilled artisan on how to overcome obstacles known in the gene therapy art. Issues such as vector targeting, adverse host immune responses directed against administered recombinant vectors, the fate and *in vivo* expression level of the transgene provided by the delivered targeting, adverse host immune responses directed against administered recombinant vectors, the fate and *in vivo* expression level of the transgene provided by the delivered vectors, routes of delivery, and others have not been addressed by the instant specification. With the lack of such guidance, it would have required undue experimentation for one skilled in the art to make and use the an *in vivo* cell comprising the vectors of the presently claimed invention.

Accordingly, due to the lack of sufficient guidance provided by the instant specification regarding to the issues set forth above, and the breadth of the claims, it would have required undue experimentation for one skilled in the art to make and use the instant broadly claimed invention.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on March 18, 2002 in Paper No. 17 (pages 14-16) have been fully considered.

With respect to the issue of ES, EC and EG cells derived from any species, Applicants argued that at the time the instant application was filed, embryonic stem (ES) cells from a variety of species including cow, hamster, pig, sheep and American mink were known in the art as evidenced by the cited articles of Saito et al., Doetschman et al., Notaranni et al. and Sukoyan et al. Additionally, the article of Pera et al. demonstrates that human EC was known at the effective filing date of the present application. Applicants further argued that the specification clearly describes the use of ES, EC and EG cells as indicated by line 28, page 3 to line 1 of page 4. Furthermore, Applicants argued that the references of Moreadith et al., Seamark and Mullins et al. cited by the Examiner address a problem associated with an *in vivo* application of ES cells and not an *in vitro* application of ES, EC or EG cells of the presently claimed invention. Applicants' arguments are found to be unpersuasive for the same reasons set forth in the Response to Arguments related to the rejection of claims 1-8, 10-16, 29-30, 32-36, 37-40, 42-44 and 46-53 above. Additionally, it is irrelevant whether ES cells to be utilized *in vitro* or *in vivo*, at the effective filing date of the present application, true non-mouse ES cells possessing the ability to contribute to the gametes to be transmitted to the next generation or to colonize the germ line (a property of a true ES cell) were not yet established.

With respect to the breadth of any replication factors, Applicants argued that the claims specified that "extrachromosomal replication of the second vector is dependent upon presence within the cell of the replication factor", and that a skilled artisan would understand that the replication factor encoded by the first vector must be specific for the replication of the second vector and therefore, it would not require undue experimentation for a skilled artisan to make and use the full breadth of the instantly claimed invention. Applicants' argument is found unpersuasive because still there is no evidence of record or in the prior arts at the effective filing date of the present application, that any non-viral replication factors can support the extrachromosomal replication of second or third vectors of the presently claimed invention.

Accordingly, claims 1-8, 10-16, 29-30, 32-36, 37-40, 42-44, 46-50 and 54 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth above.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-8, 10-16, 18, 33-40, 42-44 and 46-50 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claims 1, 33, 47 and their dependent claims, it is unclear what is encompassed by the phrase "otherwise obtaining a cell that expresses or will express the replication factor" in step (a) of the claims. Does a cell refer to an ES cell, an EC cell or an EG cell or any other cells? Additionally, it is unclear what material or method steps are required

for such a cell to express the replication at any time in the future. As such, the metes and bounds of the claims are not clearly determined.

In claim 1 and its dependent claims, the term "expressing" in step (c) is not an active step. It does not indicate the method steps required for expressing the second DNA. If the expression of the second DNA is the result of step (b), then it should be recited to reflect as such.

In claim 18, the phrase "The vector according to claim 17, wherein the vector is a viral replication factor" is unclear. How can a vector is a viral replication factor? These are two independent entities having different chemical and structural compositions. The metes and bounds of the claim are not clearly determined.

In claim 35, the phrase "the effect of simultaneous presence in the cell of a first test factor and a second test factor" is unclear. How can both a first test factor and a second test factor be present in the cell when the method as claimed comprises a single step (b) of transfecting the cell with a second vector containing a DNA coding for the protein or polypeptide or other product of DNA expression that is operably linked to a promoter.

In claim 37 and its dependent claims, it is unclear what is encompassed by the phrase "(a) a DNA sequence under investigation, linked to (b) a DNA coding for the cell active protein". Is it chemically, physically, covalently or non-covalently linked? Clarification is requested because the metes and bounds of the claims are not clearly determined.

Claim 38 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: steps involving in determining whether the cell active protein is transported to the cell surface and remains there or is secreted by the cell.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

New claim 54 is rejected under 35 U.S.C. 102(a) as being anticipated by Ramage et al. (Gene 197:83-89, 1997, IDS).

Ramage et al. teach a cultured recombinant A549 EBNA-1+ cell line supporting the episomal maintenance and replication of the pDR2 plasmid (Clonetech) that does not express a replication factor or a fragment or portion thereof (page 86, col. 2, sections 3.3 and 3.4).

Therefore, Ramage et al. anticipate the instant claim.

Claims 1-5, 8, 10-12 are rejected under 35 U.S.C. 102(b) as being anticipated by Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, IDS).

Gassmann et al. disclosed mouse ES clone 1.19 cells that contain episomal pMGD20neo DNA and express the polyoma large T antigen. These clonal cells further support the replication, episomal maintenance and expression of hygromycin B under the control of the phosphoglycerate kinase promoter of a second plasmid PGKhphΔLT20. The second plasmid also contains the polyoma *ori*, and a large T gene with a 1249-bp deletion in the coding sequence (page 1295, column 1, see entire first paragraph). For further details on the disclosed pMGD20neo and PGKhphΔLT20 vector plasmids, please refer to Fig. 1. Because the PGKhphΔLT20 vector plasmid still contains a functional endogenous T gene promoter operatively linked to a DNA sequence encoding the large T segment with a large deletion in its coding sequence (not a selectable marker), the mRNA message for this mutant large T antigen and its mutant encoded gene product is still expressed in the transfected cells. Therefore, the teachings of Gassmann et al. still meet all the recited elements in the claims, and thus the reference anticipates the instant claims.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on March 18, 2002 in Paper No. 17 (pages 19-20) have been fully considered.

Applicants mainly argued that Gassmann does not teach a second vector that contains a second DNA with a promoter for expression of the second DNA wherein the second DNA does not code for a selectable marker. Applicants' argument is not found persuasive because the PGKhphΔLT20 vector plasmid still contains a functional

endogenous T gene promoter (a second promoter) operatively linked to a DNA sequence encoding the large T segment with a large deletion in its coding sequence (not a selectable marker), the mRNA message for this mutant large T antigen and its mutant encoded gene product is still expressed in the transfected cells. Therefore, the instant claims still read over the teachings of Gassman et al.

Accordingly, claims 1-5, 8 and 10-12 are rejected under 35 U.S.C. 102(b) as being anticipated by Gassmann et al. for the reasons set forth above.

Claim Rejections - 35 USC § 103

Claims 1, 6-7, 17-24, 27, 29-30, 32 and 51-54 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, IDS).

With respect to the enabled scope of the present invention, Gassmann et al. disclosed mouse ES clone 1.19 cells that contain episomal pMGD20neo DNA and express the polyoma large T antigen. These clonal cells further support the replication, episomal maintenance and expression of hygromycin B under the control of the phosphoglycerate kinase promoter of a second plasmid PGKhphΔLT20. The second plasmid also contains the polyoma *ori*, and a large T gene with a 1249-bp deletion in the coding sequence (page 1295, column 1, see entire first paragraph). For further details on the disclosed pMGD20neo and PGKhphΔLT20 vector plasmids, please refer to Fig. 1. Upon selection for hygromycin B-resistant transfected clones, a high yield of transfected ES 1.19 cells was obtained and this high yield was attributed to the ability of

PGKhphΔLT20 to replicate and be maintained as an episome in clone 1.19 cells (page 1295, col. 1, first full paragraph). Gassmann et al. do not specifically teach that the second plasmid vector containing a second DNA sequence coding for a gene product that is not a selectable marker and that it is operably linked to a promoter for the expression of the gene product in the transfected cells, and that the second vector does not express the replication factor or a fragment or portion of the replication factor. Gassmann et al. also did not specifically teach an expression system wherein three independent vectors with recited limitations were introduced into a cell. However, Gassmann et al. specifically teach that plasmid(s) containing a polyoma ori, a selectable marker and any other gene(s) of interest could be established and maintained in transfected cells with preexisted or established presence of polyoma large T, e.g., mouse ES clone 1.19 cells that contain episomal pMGD20neo. The capability to establish plasmids as episomes in ES cells should find utility for a variety of studies of gene regulation in these cells (see page 1296, col. 1, bottom of second full paragraph).

Accordingly, it would have been obvious and within the scope of skill for a person of ordinary skill in the art at the time of invention was made to modify the disclosed method taught by Gassmann et al. by transfecting vectors (second and third or more vectors) containing a polyoma *ori* for episomal replication and maintenance, a different selection marker for selection purpose, and a gene of interest operably linked to a promoter for expression in ES clone 1.19 cells previously transfected with pMGD20neo plasmid to arrive at the instant invention. One of ordinary skilled in the art would have been motivated to carry out the above modification because Gassmann et al. suggested

that the capability to establish plasmids as episomes in ES cells should find utility for a variety of studies of gene regulation in these cells, and thereby requires the expression of genes of interest present in the transfecting vectors (see page 1296, col. 1, bottom of second full paragraph). Furthermore, with respect to the limitations recited for the vector of the present invention, it would have been obvious and within the scope of skill for an ordinary skilled artisan to modify the disclosed PGKhphΔLT20 vector plasmid of Gassmann et al. by incorporating a DNA sequence that encodes for a non-marker gene product operably linked to a promoter in the vector plasmid at the expense of the modified polyoma large T antigen encoding DNA sequence, because this mutant polyoma large T antigen is not required for the maintenance or replication of the second vector in transfected cells with preexisted or established presence of intact polyoma large T antigen. One of ordinary skilled artisan would have been motivated to carry out the above modification because Gassmann et al. specifically teach that plasmid(s) containing a polyoma ori, a selectable marker and any other gene(s) of interest (the modified plasmid vector) could be established and maintained in transfected cells with preexisted or established presence of polyoma large T, e.g., mouse ES clone 1.19 cells that contain episomal pMGD20neo plasmid, and that the capability to establish plasmids as episomes in ES cells should find utility for a variety of studies of gene regulation in these cells.

Thus, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on March 18, 2002 in Paper No. 17 (pages 21-23) have been fully considered.

Applicants argued that Gassmann does not teach a vector that does not express the replication factor or a fragment or a portion of the replication factor. Gassmann merely deleted 1249 bp of the large T gene coding sequence, and that a large portion of large T antigen remained in the Gassmann second vector. Applicants also argued that there is no reasonable expectation of success because the second vector taught by Gassmann could not be used to express a second DNA due to its large size. Applicants further argued that subsequent work by the same author teaches away from a two vector system for the expression of a heterologous gene or DNA sequence of interest as evidenced by the article of Camenisch et al. that explicitly teaches a one vector episomal system to express a heterologous gene or DNA sequence of interest in ES cells. Applicants' arguments are found to be unpersuasive for the following reasons.

Firstly, this is a 103(a) rejection, therefore Gassmann does not have teach every element of the claims. Secondly, an ordinary skilled artisan in the art has the ability to think and to make the above modifications based on the teachings and motivations provided by Gassmann. Particularly, an ordinary skilled artisan would have been motivated to delete the entire mutated large T gene coding sequence in the second PGKhphΔLT20 vector plasmid because this mutant polyoma large T antigen is not required for the maintenance or replication of the second vector in transfected cells with preexisted or established presence of intact polyoma large T antigen, and especially

since Gassmann clearly teaches that that plasmid(s) containing a polyoma ori, a selectable marker and any other gene(s) of interest could be established and maintained in transfected cells with preexisted or established presence of polyoma large T for a variety of studies of gene regulation in these cells, including mouse ES cells. As such, size of the modified vector would not be an issue and a reasonable expectation of success would be expected. Thirdly, there is nothing in the article of Camenisch et al. indicating or suggesting negatively about a two vector system for the expression of a heterologous gene or DNA sequence of interest as asserted by Applicants. Applicants are invited to point out specifically the line numbers, page numbers in the reference of Carmenisch et al. that teaches away from the two or more vector system taught by Gassmann.

Accordingly, claims 1, 6-7, 17-24, 27, 29-30, 32 and 51-54 are rejected for the reasons set forth above.

Claims 1 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, IDS) in view of Cooper (U.S. Patent No. 5,624,820).

The teachings of Gassmann et al. have been discussed above. However, Gassmann et al. do not specifically teach the use of an inducible promoter in their disclosed episomal plasmid vector system. At the time of the present invention, Cooper teaches an episomal plasmid vector system in human cells, wherein the episomal

plasmid can contain an inducible promoter such as a metallothionein promoter (col. 11, lines 34-48).

Accordingly, it would have been obvious for an ordinary skilled artisan to modify the episomal plasmid vector system taught by Gassmann et al. by introducing an inducible promoter into the episomal plasmid as taught by Cooper. One of ordinary skilled in the art would have been motivated to carry out the above modification in order to have a control in the expression of genes of interest to study their involvement in the differentiation of mouse ES cells *in vitro* as suggested by Gassmann et al. (page 1296, col. 2, first paragraph).

Therefore, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on March 18, 2002 in Paper No. 17 (page 23) have been fully considered.

Applicants argued that Cooper merely teaches the use of an inducible promoter in an episomal plasmid expression system, and that Cooper does nothing to cure the deficiencies of Gassmann. Applicants' argument is found unpersuasive for the same reasons already stated in the response to the rejection of claims 1, 6-7, 17-24, 27, 29-30, 32 and 51-54 above.

Claims 1 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, IDS) in view of Carstens et al. (Gene 164:195-202, 1995).

The teachings of Gassmann et al. have been discussed above. However, Gassmann et al. do not specifically teach the use of a site specific recombinase to regulate the transcription of the DNA in their disclosed episomal vector system. At the time of the present invention, Carstens et al. teach the use of *lox*P sites in their episomal vector system for rapid manipulation of the recovered vectors without the use of restriction enzymes (See abstract).

Accordingly, it would have been obvious for an ordinary skilled artisan to modify the episomal plasmid vector system taught by Gassmann et al. by introducing *lox*P sites into the episomal plasmid vector as taught by Carstens et al., such that the transcription of the DNA in the vector can be activated in the presence of *Cre* recombinase. One of ordinary skilled in the art would have been motivated to carry out the above modification in order to have a better control in the expression of genes of interest to study their involvement in the differentiation of mouse ES cells *in vitro* as suggested by Gassmann et al. (page 1296, col. 2, first paragraph).

Therefore, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on March 18, 2002 in Paper No. 17 (pages 23-24) have been fully considered.

Applicants argued that Carstens refers to the LoxP site in their episomal vector system, however Carstens conducted their experiments in the immortalized BB-5 cell line derived from human fibroblasts and therefore there is no reasonable expectation of success that a site specific recombinase would work in the ES cell system. Additionally, Carstens does nothing to cure the deficiencies of Gassmann. Applicants' arguments are found unpersuasive for the same reasons already stated in the response to the rejection of claims 1, 6-7, 17-24, 27, 29-30, 32 and 51-54 above. With respect to the lack of expectation of success, there is no factual evidence or any scientific reasoning provided by Applicants to indicate why there should be no expectation of success in the ES cell system. Moreover, Examiner noted that Applicants have not even conducted the claimed invention in any cells, let alone in the ES cell system.

Claims 17-18, 20-21, 23-25, 27, 29-30, 32-36 and 50-54 are rejected under 35 U.S.C. 103(a) as being unpatentable over Carstens et al. (Gene 164:195-202, 1995) in view of Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, PTO-1449 in paper no. 7) and Sambrook et al. (Molecular Cloning: A laboratory manual, second edition, 1989, pages 16.8-16.9).

With respect to the enabled scope of the present invention, Carstens et al. disclose a system that allows functional cloning of regulatory genes by the expression of

libraries of cDNA inserts either in the sense or antisense direction using Epstein-Barr virus-based expression vectors. The disclosed system is designed to identify genes that are part of or act upon the anchorage signal transduction pathway. The system is comprised of two components (a) the library expression vectors, CMV-EL and C1E-EL, containing EB*ori*P for replication in EPNA-1 expressing cells; and (b) the EPNA-1-producing cell line BB-5, a derivative of the immortalized, non-tumorigenic and anchorage dependent human fibroblast cell line, MSU1.1. BB-5 cells supported the episomal replication of CMV-EL and C1E-EL and allowed the recovery of the vector from Hirt lysates of transfected BB-5 cells (See abstract). The vectors do not contain the EBNA-1 gene, but they comprise the sequence of hygromycin B (Hy^R) marker driven by the HSV-TK minimal promoter (See Fig. 1 and page 198, lines 4-6). Transcription of a cDNA insert in the multiple cloning site (MCS) is driven either by the cytomegalovirus (CMV) immediate early promoter/enhancer (CMV-EL) or by the CMV immediate early promoter/enhancer containing an additional bp of first exon sequences of the CMV immediate early gene (C1E-EL). Different cDNA inserts can be inserted into the vectors. Carstens et al. further stated that C1E-EL was specifically designed to allow a high level transcription of mRNA from cDNA libraries inserted in the antisense orientation (page 198, column 1, lines 16-18 of the first full paragraph). Both vector constructs have been tested by inserting a luciferase cDNA into the MCS (page 198, column 1, lines 18-20 of the first full paragraph). Additionally, Carstens et al. taught the clone BB-5 was derived from the transfection of human fibroblast MSU1.1 cells with an EBNA-1 expressing vector that is presumed to contain a cryptic promoter that regulates

EBNA-1 expression (page 199, column 1, lines 8-16 and Fig. 2). Carstens et al. do not specifically teach the replication factor supplied in *trans* is polyoma large T antigen or papilloma virus replication factor. Nor do Carstens et al. specifically teach an *in vitro* assay performed in an ES cell, an EC cell or an EG cell.

However, at the effective filing date of the present application, Sambrook et al. teach that plasmid vectors bearing the viral replicons are replicated episommally as long as the appropriate trans-acting factors are provided by genes either carried on the plasmid or within the genome of the host cell (page 16.8). Sambrook et al. further teach that plasmid vectors containing replicons from papovavirues such as SV40 or polyomavirus or replicons from viruses such as bovine papillomavirus and Epstein-Barr virus have been used to propagate episomally. Gassmann et al. disclose mouse ES clone 1.19 cells that contain episomal pMGD20neo DNA and express the polyoma large T antigen. These clonal cells further support the replication, episomal maintenance and expression of hygromycin B under the control of the phosphoglycerate kinase promoter of a second plasmid PGKhphΔLT20. The second plasmid contains the polyoma *ori*. For further details on the disclosed pMGD20neo and PGKhphΔLT20 vector plasmids, please refer to Fig. 1. Upon selection for hygromycin B-resistant transfected clones, a high yield of transfected ES 1.19 cells was obtained and this high yield was attributed to the ability of PGKhphΔLT20 to replicate and be maintained as an episome in clone 1.19 cells (page1295, col. 1, first full paragraph). Gassmann et al. also specifically teach that plasmid(s) containing a polyoma *ori*, a selectable marker and any other gene(s) of interest could be established and maintained in transfected cells with preexisted or

established presence of polyoma large T, e.g., mouse ES clone 1.19 cells that contain episomal pMGD20neo. The capability to establish plasmids as episomes in ES cells should find utility for a variety of studies of gene regulation in these cells (see page 1296, col. 1, bottom of second full paragraph).

Accordingly, it would have been obvious and within the scope of skill for an ordinary skilled artisan to modify the expression vector system taught by Carstens et al. and their method of screening a library of cDNAs by replacing the Epstein-Barr based shuttle vectors requiring the Ebo*ri*P and the viral gene product EBNA-1 supplied in *trans* with an equivalent expression vector system that is dependent on other viral replication factors for the episomal propagation of the expression vectors based on the teachings of Sambrook et al. One such modified expression vector systems is one that requires the polyoma *ori* and the polyoma large T antigen supplied in *trans* as taught by Gassmann et al. Additionally, the modified expression vector system would also be applicable with a reasonable expectation of success in mouse ES cells as mouse ES cells containing episomal pMGD20neo DNA and expressing the polyoma large T antigen (supplied in *trans*) have been shown to support the replication, episomal maintenance and expression of hygromycin B of a second plasmid PGKhphΔLT20 containing the polyoma *ori*. One of ordinary skilled artisan would also have been motivated not to include any portion of large T antigen encoded region remained in the second plasmid PGKhphΔLT20 because the remaining mutant polyoma large T antigen is not required for the maintenance or replication of the second vector in transfected cells with preexisted or established presence of intact polyoma large T antigen, and

especially since Gassmann clearly teaches that that plasmid(s) containing a polyoma ori, a selectable marker and any other gene(s) of interest could be established and maintained in transfected cells with preexisted or established presence of polyoma large T for a variety of studies of gene regulation in these cells, including mouse ES cells. One of ordinary skilled artisan would have been motivated to carry out the above modification simply based on a designer's experimental choice of utilizing the viral transcription factors supplied in trans to support the episomal replication of the expression vector plasmids whose expression is unaffected by integration into host genomic DNA (see Carstens et al., page 196, col. 2, last paragraph).

Accordingly, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Claims 17 and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Carstens et al. (Gene 164:195-202, 1995) in view of Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, PTO-1449 in paper no. 7) and Sambrook et al. (Molecular Cloning: A laboratory manual, second edition, 1989, pages 16.8-16.9) as applied to claims 17-18, 20-21, 23-25, 27, 29-30, 32-36 and 50-54 above, and further in view of Cooper (U.S. Patent No. 5,624,820).

The combined teachings of Carstens et al., Sambrook et al. and Gassmann et al. have been discussed above. However, none of the reference specifically teaches that the utilized vectors contain an inducible promoter. At the time of the present invention, Cooper teaches an episomal plasmid vector system in human cells, wherein the

episomal plasmid can contain an inducible promoter such as a metallothionein promoter (col. 11, lines 34-48).

Accordingly, it would have been obvious for an ordinary skilled artisan to modify the episomal plasmid vectors in the modified expression vector system resulting from the combined teachings of Carstens et al., Sambrook et al. and Gassmann et al. by introducing an inducible promoter into the disclosed episomal plasmids as taught by Cooper. One of ordinary skilled in the art would have been motivated to carry out the above modification in order to have a better control in the expression of the genes of interest in the vectors, cDNA inserts or antisense constructs.

Therefore, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on March 18, 2002 in Paper No. 17 (pages 25-26) have been fully considered.

Applicants argued that Carstens refers to screening a cDNA library using a single vector that express a EboriP in BB-5 cells that express EBNA-1, and that Carstens do not teach an *in vitro* performed in ES, EC or EG cells. Applicants further argued that the second vector taught by Gassmann is not capable of expressing a gene of interest because of its size, and that both Cooper and Carstens teach that to successfully express a foreign gene or sequence of interest a cell line with a genomically integrated replication factor is necessary. Applicants' arguments are found to be unpersuasive

because in response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In addition, with respect to the size of the second vector of Gassmann, it would have been obvious and within the scope of skill for an ordinary skilled artisan in the art to delete entirely any large T antigen encoded region remained in the second plasmid PGKhphΔLT20 because the remaining mutant polyoma large T antigen is not required for the maintenance or replication of the second vector in transfected cells with preexisted or established presence of intact polyoma large T antigen, and especially since Gassmann clearly teaches that that plasmid(s) containing a polyoma *ori*, a selectable marker and any other gene(s) of interest could be established and maintained in transfected cells with preexisted or established presence of polyoma large T for a variety of studies of gene regulation in these cells. As such, Gassmann et al. do not teach a need for the presence of a replication factor encoded region in the second vector in transfected cells with preexisted or established presence of polyoma large T. There is no specific teaching in either Cooper or Carstens that a successful expression of a foreign gene or sequence of interest would not be accomplished in a cell line in which a replication factor is not genomically integrated in the host cell. The expression of a foreign gene or sequence of interest is not dependent on whether the replication factor is genomically integrated in the host cell or not, but rather whether a plasmid containing the foreign gene or sequence of interest can be maintained and replicated

episomally and that the foreign gene or sequence of interest is operably linked to a functional promoter. Gassmann et al. clearly showed that the second plasmid PGKhphΔLT20 can be maintained and replicated episomally in ES 1.19 cells that contain episomal pMGD20neo DNA and expressing and supplying the polyoma large T antigen in *trans*. Finally, with respect to the reference of Carstens et al., Carstens et al. clearly teach a need to construct an EBNA-1 expressing vector (another vector) in order for the transfected cell line to supply EBNA-1 in *trans* to support the maintenance and replication of the library expression vectors (page 199, column 1, lines 8-16 and Fig. 2).

Accordingly, claims 17 and 26 are rejected under 35 U.S.C. 103(a) for the reasons set forth above.

Claims 37-40 and 44-50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tashiro et al. (Science 261:600-603, 1993, PTO-1449 in paper no. 7) in view of Carstens et al. (Gene 164:195-202, 1995), Sambrook et al. (Molecular Cloning: A laboratory manual, second edition, 1989, pages 16.8-16.9), Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, PTO-1449 in paper no. 7), Williams et al. (Nature 336:684-687, 1988) and Moreau et al. (Nature 336:690-692, 1988).

With respect to the enabled scope of the instant claimed invention, Tashiro et al. teach a method for trapping signal sequences as a strategy for cloning cDNA for growth factors and type I integral membrane proteins, taking advantage of the specific NH_2-terminal signal sequences that most precursors for secreted factors and transmembrane molecules carry and that are within 400 base pairs of the 5' termini of

the mRNA without the use of specific functional assays (page 600, column 2, first full paragraph). Tashiro et al. teach the construction of the pcDL-SRα-Tac(3') vector that could direct the cell surface expression of Tac (α chain of the human interleukin-2 receptor) fusion proteins when inserts with encoded signal sequences were cloned in-frame with the correct orientation. The fusion protein expressed on plasma membranes was detected by antibodies to Tac antigen expressed on the surface of transfected COS-7 cells (See Fig. 1). The pcDL-SRα-Tac(3') vector has cloning sites between the SRα promoter and the coding sequence without a signal sequence of Tac cDNA (page 600, column 3, lines 3-10). Using this approach, two cDNAs that encode putative cytokine molecules, stromal cell-derived factor-1α (SDF-1α) and SDF-1β were cloned. Tashiro et al. do not teach the expression of the composite DNA in an ES cell, an EC cell or an EG cell; nor do they teach a system wherein the expressed cell active protein inhibits differentiation of the transfected cell and in the absence of said cell active protein the cell will differentiate. Tashiro et al. also do not teach a system using a vector expression system with limitations recited in claim 47.

However, at the effective filing date of the present application Carstens et al. disclose a system that allows functional cloning of regulatory genes by the expression of libraries of cDNA inserts using Epstein-Barr virus-based shuttle vectors (see abstract). The EBV-based shuttle vector system taught by Carstens et al. is applicable to most mammalian cells with the exception of rodent cells (page 196, column 2, lines 1-2 of the second full paragraph). Carstens et al. also teach the use of shuttle vectors offers a number of advantages over other strategies, including, (a) easy recovery of the library

vectors from selected clones, (b) expression level of the cDNA expression cassette is unaffected by integration, and (c) no cDNA expression cassettes are lost due to disruption of the vector following integration into genomic DNA (page 196, column 1, last paragraph continues to the top of column 2). Sambrook et al. teach that plasmid vectors bearing the viral replicons are replicated episommally as long as the appropriate trans-acting factors are provided by genes either carried on the plasmid or within the genome of the host cell (page 16.8). Sambrook et al. further teach that plasmid vectors containing replicons from papovavirues such as SV40 or polyomavirus or replicons from viruses such as bovine papillomavirus and Epstein-Barr virus have been used to propagate episomally. Gassmann et al. also teach a polyoma virus-based shuttle vector system in which extrachromosomal plasmid vectors can be maintained in mouse ES cells as already discussed above. Gassmann et al. noted that by analogy with bacteria, the establishment of episomal vectors in ES cells, particularly ones containing DNA segments of interest, may prove to be useful for analyzing and modifying gene expression in ES cells during embryonic development and possibly in the germ line (page 1292, col. 1, last sentence of first full paragraph). Williams et al. taught that in the presence of a secreted leukemia inhibitory factor (LIF), mouse ES cells retain the stem cell phenotype of compact colonies of small cells with a large nuclear to cytoplasmic ratio *in vitro*, whereas ES cells maintained in normal culture medium without LIF differentiate into colonies containing large, flat differentiated cells over a period of 3-6 days (page 684, column 2, last paragraph continues to top of column 1, page 685). Moreau et al. disclosed the complete cDNA sequence for a secreted LIF (Fig. 1).

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to modify the signal sequence trap method disclosed by Tashiro et al. for cloning secreted proteins and type I membrane proteins by expressing the composite DNA in a polyoma virus-based shuttle vector system in mouse ES cells, in which a DNA coding for a leukemia inhibitory factor (LIF) is used to trap NH2-termainal signal encoded sequences based on the combined teachings of Carstens et al., Sambrook et al., Gassmann et al., Williams et al. and Moreau et al. One of ordinary skill in the art would have been motivated to carry out the above modification because instead of conducting the signal sequence trap method in COS-7 cells, the polyoma virus-based shuttle vector system in mouse ES cells taught by Gassmann et al. offers various advantages as already noted by Carstens et al. for a similar Epstein-Barr virus based shuttle vector system. These advantages include a) easy recovery of the library vectors from selected clones, (b) expression level of the cDNA expression cassette is unaffected by integration, and (c) no cDNA expression cassettes are lost due to disruption of the vector following integration into genomic DNA (page 196, column 1, last paragraph continues to the top of column 2). Moreover, unlike the Epstein-Barr virus based shuttle vector system, the polyoma virus-based shuttle vector system is applicable to both mammalian cells and rodent cells (e.g., mouse ES cells). Sambrook et al. already teach that it is known in the art that plasmid vectors bearing the viral replicons are replicated episommally as long as the appropriate trans-acting factors are provided by genes either carried on the plasmid or within the genome of the host cell (page 16.8). One of ordinary skilled artisan would also have been motivated not to

include any portion of large T antigen encoded region remained in the second plasmid PGKhphΔLT20 disclosed by Gassmann et al. because the remaining mutant polyoma large T antigen is not required for the maintenance or replication of the second vector in transfected cells with preexisted or established presence of intact polyoma large T antigen, and especially since Gassmann clearly teaches that that plasmid(s) containing a polyoma *ori*, a selectable marker and any other gene(s) of interest could be established and maintained in transfected cells with preexisted or established presence of polyoma large T for a variety of studies of gene regulation in these cells, including mouse ES cells. Furthermore, the above modified method would allow an easy identification or selection of a mouse ES cell containing a DNA sequence coding for a signal polypeptide on the basis of its induced morphological or proliferative change due to the presence or absence of secreted LIF as taught by Williams et al., and Moreau et al.

Thus, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on March 18, 2002 in Paper No. 17 (pages 26-27) have been fully considered.

Applicants mainly argued that the documents cited by the Examiner do not provide a reasonable expectation of success. None of the cited documents demonstrates expression of differentiation inhibitor in ES cells or demonstrates an

episomal expression of any protein in an ES cell. The vectors discussed in Gassmann were not suitable for expression of cloned genes. Applicants' arguments are respectfully found to be unpersuasive for the following reasons.

Firstly, in response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Examiner would like to point out leukemia inhibitory factor (LIF) is a differentiation inhibitor in mouse ES cells since Williams et al. teach that in the presence of a secreted leukemia inhibitory factor (LIF), mouse ES cells retain the stem cell phenotype of compact colonies of small cells with a large nuclear to cytoplasmic ratio *in vitro*, whereas ES cells maintained in normal culture medium without LIF differentiate into colonies containing large, flat differentiated cells over a period of 3-6 days (page 684, column 2, last paragraph continues to top of column 1, page 685).

Secondly, with respect to the issue that the vectors discussed in Gassmann were not suitable for expression of cloned genes mainly because of the size of the second plasmid PGKhphΔLT20, it is obvious and within the scope of skill for an ordinary skilled artisan in the art to delete entirely the large T antigen encoded region remained in the second plasmid PGKhphΔLT20 because the remaining mutant polyoma large T antigen is not required for the maintenance or replication of the second vector in transfected cells with preexisted or established presence of intact polyoma large T antigen, and especially since Gassmann clearly teaches that that plasmid(s) containing a polyoma

ori, a selectable marker and any other gene(s) of interest could be established and maintained in transfected cells with preexisted or established presence of polyoma large T for a variety of studies of gene regulation in these cells. Clearly, Gassmann et al. do not teach a need for the presence of a replication factor encoded region in the second vector in transfected cells with preexisted or established presence of polyoma large T. Therefore, the size of the modified second vector of Gassmann should not be an issue, and in the absence of evident the contrary a reasonable expectation of success would be attained.

Accordingly, claims 37-40 and 44-50 are rejected under 35 U.S.C. 103(a) for the reasons set forth above.

Conclusions

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, Dave Nguyen, may be reached at (703) 305-2024, or SPE, Irem Yucel, Ph.D., at (703) 305-1998.

Any inquiry of a general nature or relating to the status of this application should be directed to Patent Analyst, Tracey Johnson, whose telephone number is (703) 305-2982.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1636.

Quang Nguyen, Ph.D.

DAVE T. NGUYEN
PRIMARY EXAMINER